"Marked Up Version"

ABSTRACT

The present invention describes the use of thio-phosphate as a novel metabolite for chemically modifying mRNA in cells. Thio-phosphate is taken up by both prokaryotic and eukaryotic cells, incorporated into rNTP pools and ultimately mRNA. This enables the in vivo modification of mRNA with nuclease resistant phosphorothioate internucleotide linkages. ~~When thio-phosphate is used as the sole source of phosphate in the culture medium it is possible to significantly increase the amount of mRNA per cells~~. Significant incorporation of thio-phosphate occurs such that RNA is significantly stabilized from degradation both in vivo and in vitro upon subsequent isolation. Thio-phosphate can be used as the sole source of phosphate in the culture medium for several generations resulting in a significant increase in the amount of mRNA per cell. ~~The modified RNA is also protected during isolation from nucleases.~~ The method should facilitate the detection and analysis of mRNA for research and diagnostic purposes. ~~By adjusting the ratio of thio-phosphate to phosphate in the culture medium it is also possible to enhance protein production.~~ To enhance protein production it is necessary to use a mixture of thio-phosphate and phosphate in the culture medium. Generally a specific ratio of thio-phosphate to phosphate results in the optimal synthesis of many proteins for a given organism. Significant increases in both native and recombinant proteins are possible with the use of thio-phosphate in the culture media. The method represents a significant advance for cell culture with the potential to not only enhance a variety of fermentation reactions but to also potentially stimulate cellular differentiation for a variety of therapeutic purposes.